

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71162 A2

- (51) International Patent Classification⁷: **A61K 41/00** (US). BUGAJ, Joseph, Edward; 2916 Kettering Drive, St. Charles, MO 63303 (US). RAJAGOPALAN, Raghavan; 13031 Vinson Court. Maryland Heights, MO 63043 (US).
- (21) International Application Number: PCT/US00/11060
- (22) International Filing Date: 26 April 2000 (26.04.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/135,060 20 May 1999 (20.05.1999) US
09/325,769 4 June 1999 (04.06.1999) US
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- (81) Designated States (*national*): AU, CA, JP.
- (84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
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- Published:**
— *Without international search report and to be republished upon receipt of that report.*
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- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*



WO 00/71162 A2

(54) Title: NOVEL CYANINE AND INDOCYANINE DYE BIOCONJUGATES FOR BIOMEDICAL APPLICATIONS

(57) Abstract: Dye-peptide conjugates useful for diagnostic imaging and therapy are disclosed. The dye-peptide conjugates include several cyanine dyes with a variety of bis- and tetrakis (carboxylic acid) homologues. The small size of the compounds allows more favorable delivery to tumor cells as compared to larger molecular weight imaging agents. The various dyes are useful over the range of 350-1300 nm, the exact range being dependent upon the particular dye. Use of dimethylsulfoxide helps to maintain the fluorescence of the compounds. The molecules of the invention are useful for diagnostic imaging and therapy, in endoscopic applications for the detection of tumors and other abnormalities and for localized therapy, for photoacoustic tumor imaging, detection and therapy, and for sonofluorescence tumor imaging, detection and therapy.

TITLE OF THE INVENTION

NOVEL CYANINE AND INDOCYANINE DYE BIOCONJUGATES FOR BIOMEDICAL APPLICATIONS

FIELD OF INVENTION

This invention relates generally to novel dye-bioconjugates for use in diagnosis and therapy. Particularly, this invention relates to novel compositions of cyanine dye bioconjugates of bioactive molecules for site-specific delivery of these agents for optical tomographic, endoscopic, photoacoustic, sonofluorescent, laser assisted guided surgery, and therapeutic purposes. More particularly, this invention relates to a method of preparation and use of cyanine dye bioconjugates for visualization and detection of tumors. This invention is also related to the method of preventing fluorescence quenching by the use of biocompatible organic solvents.

BACKGROUND OF THE INVENTION

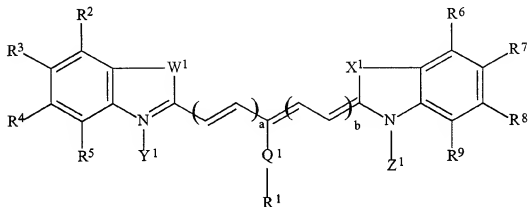
Several dyes that absorb and emit light in the visible and near-infrared region of the electromagnetic spectrum are currently being used for various biomedical applications due to their biocompatibility, high molar absorptivity, or high fluorescence quantum yields. This high sensitivity parallels that of nuclear medicine and permits visualization of organs and tissues without the negative effect of ionizing radiation. Most dyes lack specificity for particular organs or tissues and, hence, these dyes must be attached to bioactive carriers such as proteins, peptides, carbohydrates, and the like to deliver the dyes to specific regions in the body. Several studies on the use of near infrared dyes and dye-biomolecule conjugates have been published (Patonay et al., 1991; Slavik, 1994 Brinkley, 1993; Lee and Woo, U.S. Patent No. 5,453,505; Hohenschuh, WO 98/48846; Turner et al., WO 98/22146; Licha et al., WO 96/17628; and Snow et al., WO 98/48838). Of particular interest is the targeting of tumor cells with antibodies or other large protein carriers as delivery vehicles (Becker, et al., 1999). Such an approach has been widely used in nuclear medicine applications, and the major advantage is the retention of a carrier's tissue specificity since the molecular volume of the dye is substantially smaller than the carrier. However, this approach does have some serious limitations in that the diffusion of high molecular weight bioconjugates to tumor cells is highly unfavorable, and is further complicated by the net positive pressure in solid tumors (Jain, 1994). Furthermore, many dyes in general, and cyanine dyes, in particular, tend to form aggregates in aqueous media that lead to fluorescence quenching. Therefore, there is a need to

prepare low molecular weight dye-biomolecule conjugates to enhance tumor detection, and to prepare novel dye compositions to preserve fluorescence efficiency of dye molecules.

The publications and other materials used herein to support the background of the invention or provide additional details respecting the practice, are incorporated herein by reference, and for convenience are respectively grouped in the appended List of References.

SUMMARY OF THE INVENTION

The present invention relates particularly to the novel composition comprising cyanine dye bioconjugates of general formula 1 wherein a and b vary from 0 to 5; W¹ and X¹ may be



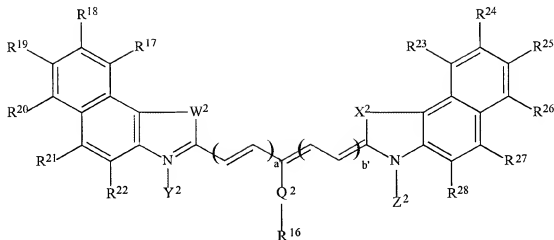
Formula 1

the same or different and are selected from the group consisting of -CR¹⁰R¹¹, -O-, -NR¹², -S-, or -Se; Q¹ is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and -NR¹³; R¹, R¹⁰ to R¹⁵, and R²⁹-R⁴⁰ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₁-C₁₀ aryl, C₁-C₁₀ alkoxy, C₁-C₁₀ polyalkoxyalkyl, -CH₂(CH₂OCH₂)_i-CH₂-OH, C₁-C₂₀ polyhydroxyalkyl, C₁-C₁₀ polyhydroxyaryl, C₁-C₁₀ aminoalkyl, -(CH₂)_d-CO₂H, -(CH₂)_e-CONH-Bm, -CH₂-(CH₂OCH₂)_f-CH₂-CONH-Bm, -(CH₂)_g-NHCO-Bm, -CH₂-(CH₂OCH₂)_h-CH₂-NHCO-Bm, -(CH₂)_y-OH or -CH₂-(CH₂OCH₂)_z-CH₂-OH; Y¹ is selected from the group consisting of -(CH₂)_r-CONH-Bm, -CH₂-(CH₂OCH₂)_j-CH₂-CONH-Bm, -(CH₂)_k-NHCO-Bm, -CH₂-(CH₂OCH₂)_l-CH₂-NHCO-Bm, -(CH₂)_m-N(R¹⁴)-(CH₂)_n-CONH-Bm, (CH₂)_{aa}-N(R²⁹)-(CH₂)_{bb}-NHCO-Bm, -(CH₂)_p-N(R¹⁵)-CH₂-(CH₂OCH₂)_q-CH₂-CONH-Bm, -(CH₂)_{cc}-N(R³⁰)-CH₂-(CH₂OCH₂)_{dd}-CH₂-NHCO-Bm, -CH₂-(CH₂OCH₂)_{ee}-CH₂-N(R³¹)-(CH₂)_f-CONH-Bm, -CH₂-(CH₂OCH₂)_{gg}-CH₂-N(R³²)-(CH₂)_{hh}-NHCO-Bm, -CH₂-(CH₂OCH₂)_{ii}-CH₂-N(R³³)-CH₂-(CH₂OCH₂)_{jj}-CH₂-CONH-Bm or -CH₂-(CH₂OCH₂)_{kk}-CH₂-N(R³⁴)-CH₂-(CH₂OCH₂)_{ll}-CH₂-NHCO-Bm; d, e, g, i, k, m, n, p, aa, bb, cc, ff, hh

and yy vary from 1 to 10; c, f, h, j, l, q, dd, ee, gg, ii, jj, kk, ll and zz vary from 1 to 100; Bm is any bioactive peptide, protein, cell, oligosaccharide, glycopeptide, peptidomimetic, drug, drug mimic, hormone, metal chelating agent, radioactive or nonradioactive metal complex, or echogenic agent; Z¹ is selected from the group consisting of -(CH₂)_i-CO₂H, -(CH₂)_i-OH, -(CH₂)_i-NH₂, -CH₂-(CH₂OCH₂)_j-CH₂-CO₂H, -CH₂-(CH₂OCH₂)_j-CH₂-OH, -CH₂-(CH₂OCH₂)_j-CH₂-NH₂, -(CH₂)_i-CONH-Dm, -CH₂-(CH₂OCH₂)_i-CH₂-CONH-Dm, -(CH₂)_i-NHCO-Dm, -CH₂-(CH₂OCH₂)_i-CH₂-NHCO-Dm, -(CH₂)_w-N(R¹⁴)-(CH₂)_x-CONH-Dm, (CH₂)_{mm}-N(R³⁵)-(CH₂)_{nn}-NHCO-Dm, -(CH₂)_l-N(R¹⁵)-CH₂-(CH₂OCH₂)₂-CH₂-CONH-Dm, -(CH₂)_{uu}-N(R³⁹)-CH₂-(CH₂OCH₂)_w-CH₂-NHCO-Dm, -CH₂-(CH₂OCH₂)_{ww}-CH₂-N(R⁴⁰)-(CH₂)_{xx}-CONH-Dm, -CH₂-(CH₂OCH₂)_{oo}-CH₂-N(R³⁶)-(CH₂)_{pp}-NHCO-Dm, -CH₂-(CH₂OCH₂)_{qq}-CH₂-N(R³⁷)-CH₂-(CH₂OCH₂)_{rr}-CH₂-CONH-Dm or -CH₂-(CH₂OCH₂)_{ss}-CH₂-N(R³⁸)-CH₂-(CH₂OCH₂)_{tt}-CH₂-NHCO-Dm; r, t, v, w, x, y, mm, nn, pp, uu and xx vary from 1 to 10, and o, s, u, z, oo, qq, rr, ss, tt, vv and ww vary from 1 to 100; and Dm is any bioactive peptide, antibody, antibody fragment, oligosaccharide, drug, drug mimic, glycomimetic, glycopeptide, peptidomimetic, hormone, and the like; R² to R⁹ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₁-C₁₀ aryl, hydroxyl, C₁-C₁₀ polyhydroxyalkyl, C₁-C₁₀ alkoxy, amino, C₁-C₁₀ aminoalkyl, cyano, nitro, or halogen.

The present invention also relates to the novel composition comprising cyanine dye bioconjugates of general formula 2 wherein a' and b' are defined in the same manner as a and b; W² and X² are defined in the same manner W¹ and X¹; Q² is defined in the same manner as Q¹; R¹⁶ is defined in the same manner as R¹; Y² is defined in the same manner as Y¹; Z² is defined in the same manner as Z¹; and R¹⁷ to R²⁸ are defined in the same manner as R².

This invention is also related to the method of preventing fluorescence quenching. It is known that cyanine dyes generally form aggregates in aqueous media leading to fluorescence quenching. This problem is further accentuated by the conjugation of large hydrophobic dyes to small molecular peptides. We observed that the addition of a biocompatible organic solvent such as 1-50% dimethylsulfoxide (DMSO) restored the fluorescence by preventing aggregation and allowed the visualization of tumors.

**Formula 2**

In one embodiment of the invention, the dye-peptide conjugates are useful for optical tomographic, endoscopic, photoacoustic and sonofluorescent applications for the detection and treatment of tumors and other abnormalities.

In another aspect of the invention, the dye-peptide conjugates of the invention are useful for localized therapy.

In yet another aspect of the invention, the dye peptide conjugates of the invention are useful for the detection of the presence of tumors and other abnormalities by monitoring the blood clearance profile of the conjugates.

In a further embodiment of the invention, the dye-peptide conjugates are useful for laser assisted guided surgery for the detection of small micrometastases of, e.g., somatostatin subtype 2 (SST-2) positive, tumors upon laparoscopy.

In yet another aspect of the invention, the dye-peptide conjugates of this invention are useful for diagnosis of atherosclerotic plaques and blood clots.

BRIEF DESCRIPTION OF THE FIGURES

The file of this patent contains at least one drawing executed in color. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figures 1A-F represent images at 2 minutes and 30 minutes post injection of indocyanine green into rats with various tumors. Figures 1A-B are images of a rat with an induced pancreatic ductal adenocarcinoma tumor (DSL 6A) imaged at 2 minutes (Figure 1A) and 30 minutes (Figure 1B) post injection. Figures 1C-D are images of a rat with an induced prostatic carcinoma tumor (R3327-H) imaged at 2 minutes (Figure 1C) and 30 minutes (Figure 1D) post injection. Figures 1E-F are images of a rat with an induced pancreatic acinar carcinoma (CA20948) expressing the SST-2 receptor imaged at 2 minutes (Figure 1E) and 30 minutes (Figure 1F) post injection.

Figures 2A-B show a comparison of the uptake of indocyanine green and Cytate 1 at 45 minutes post injection in rats with the pancreatic acinar carcinoma (CA20948).

Figures 3A-B show images of rats with the pancreatic acinar carcinoma (CA20948) 90 minutes (Figure 3A) and 19 hours (Figure 3B) post injection of Cytate 1.

Figures 4A-B show images comparing selected tissue parts of a rat with the pancreatic acinar carcinoma (CA20948) 22 hours post injection with Cytate 1 (Figure 4A) and the same tissue parts imaged in an uninjected rat (Figure 4B).

Figure 5 is an image of individual organs taken from a rat with pancreatic acinar carcinoma (CA20948) about 24 hours after injection with Cytate 1.

Figure 6 is the clearance profile of Cytate 1 from the blood of a normal rat monitored at 830 nm after excitation at 780 nm.

Figure 7 is the clearance profile of Cytate 1 from the blood of a CA20948 tumored rat monitored at 830 nm after excitation at 780 nm.

Figure 8 is the clearance profile of Cytate 2 from the blood of a normal rat monitored at 830 nm after excitation at 780 nm.

Figure 9 is the clearance profile of Cytate 2 from the blood of a CA20948 tumored rat monitored at 830 nm after excitation at 780 nm.

Figure 10 is the clearance profile of Cytate 4 from the blood of a normal rat monitored at 830 nm after excitation at 780 nm.

DETAILED DESCRIPTION OF THE INVENTION

The novel bioconjugates of the present invention exploit the symmetric nature of the cyanine and indocyanine dye structures by incorporating one to ten receptor targeting groups, in close proximity to each other such that the receptor binding can be greatly enhanced due to a cooperative

effect. Accordingly, several cyanine dyes containing one or more targeting domains have been prepared and tested *in vivo* for biological activity.

The novel compositions of the present invention comprise dye-bioconjugates of formulas 1 and 2 and offer significant advantages over those currently described in the art. They are applicable to various biomedical applications including, but not limited to, tomographic imaging of organs; monitoring of organ functions; coronary angiography; fluorescence endoscopy; detection, imaging, and therapy of tumors; laser guided surgery, photoacoustic and sonofluorescent methods; and the like. Specific embodiments to accomplish some of the aforementioned biomedical applications are given below. The bioconjugates are prepared by the methods well known in the art and are shown in Schemes (1-3).

In a preferred embodiment, the bioconjugates according to the present invention have the general formula 1 wherein a and b vary from 0 to 3; Q¹ is a single bond; R¹ to R⁹ are hydrogens; W¹ and X¹ are -CR¹⁰R¹¹; Y¹ is -(CH₂)_i-CONH-Bm, -(CH₂)_i-NHCO-Bm, or -(CH₂)_m-N(R¹⁴)-(CH₂)_n-CONH-Bm; Z¹ is -(CH₂)_r-OH, -(CH₂)_r-CO₂H, -(CH₂)_r-NH₂, -(CH₂)_t-CONH-Dm, -(CH₂)_t-NHCO-Dm, -(CH₂)_u-N(R¹⁴)-(CH₂)_x-CONH-Dm, -CH₂-(CH₂OCH₃)₅-CH₂-OH, -CH₂-(CH₂OCH₂)₅-CH₂-CO₂H, -CH₂-(CH₂OCH₂)₅-CH₂-NH₂, -CH₂-(CH₂OCH₂)_u-CH₂-CONH-Dm, -CH₂-(CH₂OCH₂)_u-CH₂-NHCO-Dm, or -CH₂-(CH₂OCH₂)_{ww}-CH₂-N(R⁴⁰)-(CH₂)_{xx}-CONH-Dm; and Bm is a tumor specific biomolecule or drug mimic selected from the group consisting of peptides or oligosaccharides containing 2-50 monomer units and including somatostatin, bombesin, neurotensin, cholecystokinin and vasoactive intestinal polypeptide.

In another preferred embodiment, the bioconjugates according to the present invention have the general formula 1 wherein a and b are 3; Q¹ is a single bond; R¹ to R⁹ are hydrogens; W¹ and X¹ are -C(CH₃)₃; Y¹ is -(CH₂)_i-CONH-Bm or -CH₂-(CH₂OCH₂)_j-CH₂-CONH-Bm wherein i varies from 1 to 4; and Z¹ is -(CH₂)_r-CO₂H, -(CH₂)_t-CONH-Dm, -CH₂-(CH₂OCH₂)₅-CH₂-CO₂H or -CH₂-(CH₂OCH₂)_u-CH₂-CONH-Dm, wherein r and t vary from 1-4; and Bm is a tumor specific biomolecule selected from the group consisting of Octreotate and its mimics, Octreotide derivatives and their mimics, bombesin analogs, cholecystokinin analogs, and neurotensin analogs.

In another preferred embodiment, the bioconjugates according to the present invention have the general formula 2 wherein a' and b' vary from 0 to 3; Q² is a single bond; R¹⁶ to R²⁸ are hydrogens; W² and X² are -CR¹⁰R¹¹; Y² is -(CH₂)₇-CONH-Bm, -(CH₂)_k-NHCO-Bm, or -(CH₂)_m-N(R¹⁴)-(CH₂)_n-CONH-Bm; Z² is -(CH₂)_r-CO₂H, -(CH₂)₇-NH₂, -(CH₂)₇-OH, -(CH₂)₇-CONH-Dm, -(CH₂)₅-NHCO-Dm, -(CH₂)₆-N(R¹⁴)-(CH₂)₅-CONH-Dm, -CH₂-(CH₂OCH₂)₅-CH₂-CO₂H, -CH₂-(CH₂OCH₂)₅-CH₂-NH₂, -CH₂-(CH₂OCH₂)₅-CH₂-OH, -CH₂-(CH₂OCH₂)₅-CH₂-CONH-Dm, -CH₂-(CH₂OCH₂)₆-CH₂-NHCO-Dm, -CH₂-(CH₂OCH₂)_{ww}-CH₂-N(R⁴⁰)-(CH₂)_{xx}-CONH-Dm; and Bm is a tumor specific biomolecule or drug mimic selected from the group consisting of peptides and oligosaccharides containing 2-50 monomer units.

In another preferred embodiment, the bioconjugates according to the present invention have the general formula 2 wherein a' and b' are 3; Q² is a single bond; R¹⁶ to R²⁸ are hydrogens; W² and X² are -C(CH₃)₃; Y² is -(CH₂)_i-CONH-Bm or -CH₂-(CH₂OCH₂)_j-CH₂-CONH-Bm wherein i varies from 1 to 4; and Z² is -(CH₂)_r-CO₂H, -(CH₂)₇-CONH-Dm, -CH₂-(CH₂OCH₂)₅-CH₂-CO₂H or -CH₂-(CH₂OCH₂)_u-CH₂-CONH-Dm, wherein r and t vary from 1-4; and Bm is a tumor specific biomolecule selected from the group consisting of Octreotate derivatives and their mimics, Octreotide derivatives and their mimics, bombesin analogs and their mimics, cholecystokinin analogs and their mimics, and neurotensin analogs and their mimics.

In a preferred embodiment, the methods utilize light of a wavelength in the region of 350-1300 nm.

In a preferred embodiment, a therapeutic procedure comprises attaching a porphyrin to a bioconjugate and using it for photodynamic therapy or shining light of a specific wavelength on the dipeptide conjugate of this invention to achieve a photodynamic therapy effect.

The compositions of the invention can be formulated into diagnostic compositions for enteral or parenteral administration. These compositions contain an effective amount of the dye along with conventional pharmaceutical carriers and excipients appropriate for the type of administration contemplated. For example, parenteral formulations advantageously contain a sterile aqueous solution or suspension of dye according to this invention. Parenteral compositions may be injected directly or mixed with a large volume parenteral composition for systemic administration. Such solutions also may contain pharmaceutically acceptable buffers and, optionally, electrolytes such as sodium chloride.

Formulations for enteral administration may vary widely, as is well known in the art. In general, such formulations are liquids which include an effective amount of the dye in aqueous

solution or suspension. Such central compositions may optionally include buffers, surfactants, thixotropic agents, and the like. Compositions for oral administration may also contain flavoring agents and other ingredients for enhancing their organoleptic qualities.

The diagnostic compositions are administered in doses effective to achieve the desired enhancement. Such doses may vary widely, depending upon the particular dye employed, the organs or tissues which are the subject of the imaging procedure, the imaging equipment being used, and the like.

The diagnostic compositions of the invention are used in the conventional manner. The compositions may be administered to a patient, typically a warm-blooded animal, either systemically or locally to the organ or tissue to be imaged, and the patient then subjected to the imaging procedure.

A combination of the above represents an important approach to the use of small molecular targeting groups to image tumors by the optical methods. The present invention is further detailed in the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below are utilized.

EXAMPLE 1

Synthesis of Bisethylcarboxymethylindocyanine Dye

(Scheme 1, R_1, R_2 = fused phenyl; $A = CH_2$, $n = 1$ and $R = R' = CO_2H$)

A mixture of 1,1,2-trimethyl-[1H]-benz[e]indole (9.1 g, 43.58 mmol) and 3-bromopropanoic acid (10.0 g, 65.37 mmol) in 1,2-dichlorobenzene (40 mL) was heated at 110°C for 12 hours. The solution was cooled to room temperature and the red residue obtained was filtered and washed with acetonitrile:diethyl ether (1:1) mixture. The solid obtained was dried under vacuum to give 10 g (64%) of light brown powder. A portion of this solid (6.0 g; 16.56 mmol), glutacanaldehyde dianil monohydrochloride (2.36 g, 8.28 mmol) and sodium acetate trihydrate (2.93 g, 21.53 mmol) in ethanol (150 mL) were refluxed for 90 minutes. After evaporating the solvent, 40 mL of a 2 N aqueous HCl was added to the residue and the mixture was centrifuged and the supernatant was decanted. This procedure was repeated until the supernatant became nearly colorless. About 5 mL of water:acetonitrile (3:2) mixture was added to the solid residue and lyophilized to obtain 2 g of dark green flakes. The purity of the compound was established with ¹H-NMR and LC-Mass spectrometry.

EXAMPLE 2

Synthesis of Bis(phenyl)carboxymethylindocyanine Dye

(Scheme 1, R_1, R_2 = fused phenyl; $A = CH_2$, $n = 4$ and $R = R' = CO_2H$)

A mixture of 1,1,2-trimethyl-[1H]-benz[e]indole (20 g, 95.6 mmol) and 6-bromohexanoic acid (28.1 g, 144.1 mmol) in 1,2-dichlorobenzene (250 mL) was heated at 110 °C for 12 hours. The green solution was cooled to room temperature and the brown solid precipitate formed was collected by filtration. After washing the solid with 1,2-dichlorobenzene and diethyl ether, the brown powder obtained (24 g, 64%) was dried under vacuum at room temperature. A portion of this solid (4.0 g; 9.8 mmol), glutaraldehyde dianil monohydrochloride (1.4 g, 5 mmol) and sodium acetate trihydrate (1.8 g, 12.9 mmol) in ethanol (80 mL) were refluxed for 1 hour. After evaporating the solvent, 20 mL of a 2 N aqueous HCl was added to the residue and the mixture was centrifuged and the supernatant was decanted. This procedure was repeated until the supernatant became nearly colorless. About 5 mL of water:acetonitrile (3:2) mixture was added to the solid residue and lyophilized to obtain about 2 g of dark green flakes. The purity of the compound was established with ¹H-NMR and LC-Mass spectrometry.

EXAMPLE 3

Synthesis of Bis(phenyl)carboxymethylindocyanine Dye

(Scheme 1, R_1, R_2 = fused phenyl; $A = CH_2$, $n = 1$ and $R = R' = CO_2H$)

This compound was prepared as described in Example 1 except that 1,1,2-trimethylindole was used as the starting material.

EXAMPLE 4

Synthesis of Bis(hexaethyleneglycol)carboxymethylindocyanine Dye

(Scheme 1, R_1, R_2 = fused phenyl; $A = CH_2OCH_2$, $n = 6$ and $R = R' = CO_2H$)

This compound was prepared as described in Example 1 except that ω-bromohexaethyleneglycolpropanoic acid was used in place of bromopropanoic acid and the reaction was carried out in 1,2-dimethoxypropane.

EXAMPLE 5

Synthesis of Bisethylcarboxymethylindocyanine Dye

(Scheme 2, R₁, R₂ = fused phenyl; A = CH₂, and n = 1)

This compound is readily prepared as described in Example 1 except that 3-bromo-1-(N,N-bis-carboxymethyl)aminopropane is used in place of bromopropanoic acid.

EXAMPLE 6

Synthesis of Peptides

The procedure described below is for the synthesis of Octreotate. Other peptides of this invention were prepared by a similar procedure with slight modifications in some cases.

The octapeptide was prepared by an automated fluorenylmethoxycarbonyl (Fmoc) solid phase peptide synthesis using a commercial peptide synthesizer from Applied Biosystems (Model 432A SYNERGY Peptide Synthesizer). The first peptide cartridge contained Wang resin pre-loaded with Fmoc-Thr on 25 μ mole scale. Subsequent cartridges contained Fmoc-protected amino acids with side chain protecting groups for the following amino acids: Cys(Acm), Thr(t-Bu), Lys(Boc), Trp(Boc) and Tyr(t-Bu). The amino acid cartridges were placed on the peptide synthesizer and the product was synthesized from the C- to the N-terminal position. The coupling reaction was carried out with 75 μ moles of the protected amino acids in the presence of 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/N-hydroxybenzotriazole (HOBt). The Fmoc protecting group was removed with 20% piperidine in dimethylformamide. After the synthesis was complete, the thiol group was cyclized with thallium trifluoroacetate and the product was cleaved from the solid support with a cleavage mixture containing trifluoroacetic acid (85%):water (5%):phenol (5%):thioanisole (5%) for 6 hours. The peptide was precipitated with t-butyl methyl ether and lyophilized with water:acetonitrile (2:3) mixture. The peptide was purified by HPLC and analyzed with LC/MS. The amino acid sequence of Octreotate is: D-Phe-Cys'-Tyr-D-Trp-Lys-Thr-Cys'-Thr (SEQ ID NO:1), wherein Cys' indicates the presence of an intramolecular disulfide bond between two cysteine amino acids.

Octreotide was prepared by the same procedure : D-Phe-Cys'-Tyr-D-Trp-Lys-Thr-Cys'-Thr-OH (SEQ ID NO:2), wherein Cys' indicates the presence of an intramolecular disulfide bond between two cysteine amino acids.

Bombesin analogs were prepared by the same procedure except that cyclization with thallium trifluoroacetate was not needed. Side-chain deprotection and cleavage from the resin was carried out with 50 μ L each of ethanedithiol, thioanisole and water, and 850 μ L of trifluoroacetic acid. Two analogues were prepared: Gly-Ser-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH₂ (SEQ ID NO:3) and
5 Gly-Asp-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH₂ (SEQ ID NO:4).

Cholecystokinin octapeptide analogs were prepared as described for Octreotate without the cyclization step. Three analogs were prepared: Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂ (SEQ ID NO:5); Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH₂ (SEQ ID NO:6); and D-Asp-Tyr-Nle-Gly-Trp-Nle-Asp-Phe-NH₂ (SEQ ID NO:7) wherein Nle is norleucine.

10 Neurotensin analog was prepared as described for Octreotate without the cyclization step: D-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu (SEQ ID NO:8).

EXAMPLE 7

Synthesis of Peptide-Dye Conjugates

15 The method described below is for the synthesis of Octreotate conjugates but a similar procedure is used for the synthesis of other peptide-dye conjugates.

The Octreotate was prepared as described in Example 6 but the peptide was not cleaved from the solid support and the N-terminal Fmoc group of Phe was retained. The thiol group was cyclized with thallium trifluoroacetate and the Phe was deprotected to liberate the free amine.
20 Bisethylcarboxymethylindocyanine dye (53 mg, 75 μ moles) was added to an activation reagent consisting of a 0.2 M solution of HBTU/HOBt in DMSO (375 μ L), and 0.2 M solution of diisopropylethylamine in DMSO (375 μ L). The activation was complete in about 30 minutes and the resin-bound peptide (25 μ moles) was added to the dye. The coupling reaction was carried out at room temperature for 3 hours. The mixture was filtered and the solid residue was washed with
25 DMF, acetonitrile and THF. After drying the green residue, the peptide was cleaved from the resin and the side chain protecting groups were removed with a mixture of 85% trifluoroacetic acid, 5% water, 5% thioanisole and 5% phenol. The resin was filtered and cold t-butyl methyl ether (MTBE) was used to precipitate the dye-peptide conjugate which was dissolved in acetonitrile:water (2:3) mixture and lyophilized. The product was purified by HPLC to give the monoOctreotate-
30 Bisethylcarboxymethylindocyanine dye (**Cytate 1**, 80%) and the bisOctreotate-Bisethylcarboxymethylindocyanine dye (**Cytate 2**, 20%). The monoOctreotate conjugate can be obtained almost exclusively (>95%) over the bis conjugate by reducing the reaction time to 2 hours.

However, this also leads to incomplete reaction and the free Octreotate must be carefully separated from the dye conjugate in order to avoid saturation of the receptors by the non-dye conjugated peptide.

Octreotate-bis(5-pentylcarboxymethyl)indocyanine dye was prepared as described above with some modifications. Bis(5-pentylcarboxymethyl)indocyanine dye (60 mg, 75 μ moles) was added to an activation reagent consisting of a 0.2 M solution of HBTU/HOBt in DMSO (400 μ L), and 0.2 M solution of diisopropylethylamine in DMSO (400 μ L). The activation was complete in about 30 minutes and the resin-bound peptide (25 μ moles) was added to the dye. The reaction was carried out at room temperature for 3 hours. The mixture was filtered and the solid residue was washed with DMF, acetonitrile and THF. After drying the green residue, the peptide was cleaved from the resin and the side chain protecting groups were removed with a mixture of 85% trifluoroacetic acid, 5% water, 5% thioanisole and 5% phenol. The resin was filtered and cold t-butyl methyl ether (MTBE) was used to precipitate the dye-peptide conjugate which was dissolved in acetonitrile:water (2:3) mixture and lyophilized. The product was purified by HPLC to give Octreotate-1,1,2-trimethyl-[1H]-benz[e]indole propanoic acid conjugate (10%), monoOctreotate-bis(5-pentylcarboxymethyl)indocyanine dye (Cytate 3, 60%) and bisOctreotate-bis(5-pentylcarboxymethyl)indocyanine dye (Cytate 4, 30%).

EXAMPLE 8

Formulation of peptide-dye conjugates in dimethyl sulfoxide (DMSO)

The dye-peptide conjugates are sparingly soluble in water and require the addition of solubilizing agents or co-solvents. Addition of 1-20% aqueous ethanol to the conjugates partially quenched the fluorescence intensity *in vitro* and the fluorescence was completely quenched *in vivo* (the conjugate was not detected by the CCD camera). Addition of 1-50% of DMSO either re-established or increased the fluorescence intensity of the conjugates *in vitro* and *in vivo*. The dye fluorescence remained intense for over one week. The DMSO formulations were well tolerated by experimental animals used for this invention.

EXAMPLE 9

Imaging of pancreatic ductal adenocarcinoma (DSL 6A) with Indocyanine Green (ICG)

A non-invasive *in vivo* fluorescence imaging apparatus was employed to assess the efficacy of contrast agents developed for tumor detection in animal models. A LaserMax Inc. laser diode of

nominal wavelength 780 nm and nominal power of 40 mW was used. The detector was a Princeton Instruments model RTE/CCD-1317-K/2 CCD camera with a Rodenstock 10 mm F2 lens (stock #542.032.002.20) attached. An 830 nm interference lens (CVI Laser Corp., part # F10-830-4-2) was mounted in front of the CCD input lens such that only emitted fluorescent light from the contrast agent was imaged. Typically, an image of the animal was taken pre-injection of contrast agent. This image was subsequently subtracted (pixel by pixel) from the post injection images. However, the background subtraction was never done once the animal had been removed from the sample area and returned at a later time for images taken several hours post injection.

DSL 6A tumors were induced in male Lewis rats in the left flank area by the introduction of material from a solid (donor) implant and the tumors were palpable in approximately 14 days. The animals were anesthetized with rat cocktail (xylazine; ketamine; acepromazine 1.5: 1.5: 0.5) at 0.8 mL/kg via intramuscular injection. The area of the tumor (left flank) was shaved to expose tumor and surrounding surface area. A 21 gauge butterfly equipped with a stopcock and two syringes containing heparinized saline was placed into the later tail vein of the rat. Patency of the vein was checked prior to administration of the ICG via the butterfly apparatus. Each animal received 500 μ L of a 0.42 mg/mL solution of ICG in water. The images obtained at 2 and 30 minutes post injection are shown in Figures 1A-B.

EXAMPLE 10

Imaging of Prostatic Carcinoma (R3327-H) with Indocyanine Green (ICG)

The imaging apparatus and the procedure used are described in Example 9. R3327-H tumors were induced in young male Copenhagen rats in the left flank area from a solid implant. These tumors grow very slowly and palpable masses were present 4-5 months post implant. The images obtained at 2 and 30 minutes post injection are shown in Figure 1C-D.

EXAMPLE 11

Imaging of Rat Pancreatic Acinar Carcinoma (CA20948) with Indocyanine Green (ICG)

The imaging apparatus and the procedure used are described in Example 9. Rat pancreatic acinar carcinoma expressing the SST-2 receptor (CA20948) were induced by solid implant technique in the left flank area and palpable masses were detected 9 days post implant. The images obtained at 2 and 30 minutes post injection are shown in Figure 1E-F.

EXAMPLE 12

Imaging of Rat Pancreatic Acinar Carcinoma (CA20948) with Cytate 1

The imaging apparatus and the procedure used are described in Example 9 except that each animal received 500 μ L of a 1.0 mg/mL solution of Cytate 1 solution of 25% dimethylsulfoxide in water. Rat pancreatic acinar carcinoma expressing the SST-2 receptor (CA20948) were induced by solid implant technique in the left flank area and palpable masses were detected 24 days post implant. Images were obtained at various times post injection. Uptake into the tumor was seen at 2 minutes but was not maximal until about 5 minutes. Figures 2A-B show a comparison of the uptake of ICG and Cytate 1 at 45 minutes in rats with the CA20948 tumor cell line. By 45 minutes the ICG has mostly cleared (Figure 2A) whereas the Cytate 1 is still quite intense (Figure 2B). This dye fluorescence remained intense in the tumor for several hours post-injection.

EXAMPLE 13

Imaging of Rat Pancreatic Acinar Carcinoma (CA20948) with Cytate 1 Compared with Imaging with ICG

Using indocyanine green (ICG), three different tumor lines were imaged optically using a CCD camera apparatus. Two of the lines, DSL 6/A (pancreatic) and Dunning R3327-H (prostate) indicated slow perfusion of the agent over time into the tumor and reasonable images were obtained for each. The third line, CA20948 (pancreatic), indicated only a slight but transient perfusion that was absent after only 30 minutes post injection. This indicates no non-specific localization of ICG into this line compared to the other two tumor lines suggesting a vastly different vascular architecture for this type of tumor (see Figures 1A-F). The first two tumor lines (DSL 6/A and R3327-H) are not as highly vascularized as CA20948 which is also rich in somatostatin (SST-2) receptors. Consequently, the detection and retention of a dye in this tumor model is an important index of receptor-mediated specificity.

Octreotate is known to target somatostatin (SST-2) receptors, hence, we prepared cyano-Octreotates (Cytate 1 and Cytate 2). Cytate 1 was evaluated in the CA20948 Lewis rat model. Using the CCD camera apparatus strong localization of this dye was observed in the tumor at 90 minutes post injection (Figure 3A). At 19 hours post injection the animal was again imaged (Figure 3B) and tumor visualization was easily observed showing specificity of this agent for the SST-2 receptors present in this tumor line. As a control, the organs were imaged again (Figure 4A) and the image was compared with that of the same tissues in the uninjected rat (Figure 4B).

Individual organs were removed and imaged. High uptake of the material was observed in the pancreas, adrenals and tumor tissue, while heart, muscle, spleen and liver indicated significantly less uptake (Figure 5). This correlates very nicely with radiolabeled Octreotate in the same model system (see de Jong et al., 1998).

EXAMPLE 14

Monitoring of the blood clearance profile of peptide-dye conjugates

A laser of appropriate wavelength for excitation of the dye chromophore was directed into one end of a fiber optic bundle and the other end was positioned a few millimeters from the ear of a rat. A second fiber optic bundle was also positioned near the same ear to detect the emitted fluorescent light and the other end was directed into the optics and electronics for data collection. An interference filter (IF) in the collection optics train was used to select emitted fluorescent light of the appropriate wavelength for the dye chromophore.

Sprague-Dawley or Fischer 344 rats were used in these studies. The animals were anesthetized with urethane administered via intraperitoneal injection at a dose of 1.35 g/kg body weight. After the animals had achieved the desired plane of anesthesia, a 21 gauge butterfly with 12" tubing was placed in the lateral tail vein of each animal and flushed with heparinized saline. The animals were placed onto a heating pad and kept warm throughout the entire study. The lobe of the left ear was affixed to a glass microscope slide to reduce movement and vibration.

Incident laser light delivered from the fiber optic was centered on the affixed ear. Data acquisition was then initiated, and a background reading of fluorescence was obtained prior to administration of the test agent. For Cytates 1 or 2, the peptide-dye conjugate was administered to the animal through a bolus injection in the lateral tail vein, typically of 0.5 to 2.0 mL. The fluorescence signal rapidly increased to a peak value. The signal then decayed as a function of time as the conjugate cleared from the bloodstream.

This procedure was repeated with several dye-peptide conjugates in normal and tumored rats and representative profiles are shown in Figures 6 to 10.

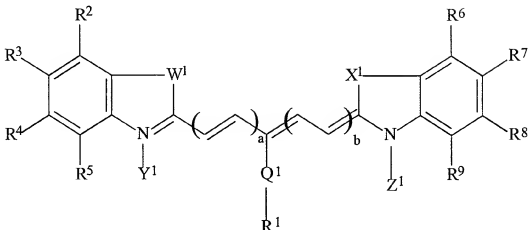
While the invention has been disclosed by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.

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- Turner J, et al. "Optical diagnostic agents for diagnosis of neurodegenerative diseases by means of near infra-red radiation (NIR radiation)", WO 98/22146.
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- Snow RA, et al., "Compounds", WO 98/48838.

WHAT IS CLAIMED IS:

1. A composition comprising cyanine dye bioconjugate of formula,



wherein a and b vary from 0 to 5; W¹ and X¹ may be the same or different and are selected from the group consisting of -CR¹⁰R¹¹, -O-, -NR¹², -S-, or -Se; Q¹ is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and -NR¹³; R¹, R¹⁰ to R¹⁵, and R²⁹-R⁴⁰ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₁-C₁₀ aryl, C₁-C₁₀ alkoxy, C₁-C₁₀ polyalkoxyalkyl, -CH₂(CH₂OCH₂)_e-CH₂-OH, C₁-C₂₀ polyhydroxyalkyl, C₁-C₁₀ polyhydroxyaryl, C₁-C₁₀ aminoalkyl, -(CH₂)_d-CO₂H, -(CH₂)_e-CONH-Bm, -CH₂-(CH₂OCH₂)_f-CH₂-CONH-Bm, -(CH₂)_g-NHCO-Bm, -CH₂-(CH₂OCH₂)_h-CH₂-NHCO-Bm, -(CH₂)_{yy}-OH or -CH₂-(CH₂OCH₂)_{zz}-CH₂-OH; Y¹ is selected from the group consisting of -(CH₂)_i-CONH-Bm, -CH₂-(CH₂OCH₂)_j-CH₂-CONH-Bm, -(CH₂)_k-NHCO-Bm, -CH₂-(CH₂OCH₂)_l-CH₂-NHCO-Bm, -(CH₂)_m-N(R¹⁴)-(CH₂)_n-CONH-Bm, (CH₂)_{aa}-N(R²⁹)-(CH₂)_{bb}-NHCO-Bm, -(CH₂)_p-N(R¹⁵)-CH₂-(CH₂OCH₂)_q-CH₂-CONH-Bm, -(CH₂)_{cc}-N(R³⁰)-CH₂-(CH₂OCH₂)_{dd}-CH₂-NHCO-Bm, -CH₂-(CH₂OCH₂)_{ee}-CH₂-N(R³¹)-(CH₂)_{ff}-CONH-Bm, -CH₂-(CH₂OCH₂)_{gg}-CH₂-N(R³²)-(CH₂)_{hh}-NHCO-Bm, -CH₂-(CH₂OCH₂)_{ii}-CH₂-N(R³³)-CH₂-(CH₂OCH₂)_{jj}-CH₂-CONH-Bm or -CH₂-(CH₂OCH₂)_{kk}-CH₂-N(R³⁴)-CH₂-(CH₂OCH₂)_{ll}-CH₂-NHCO-Bm; d, e, g, i, k, m, n, p, aa, bb, cc, ff, hh and yy vary from 1 to 10; c, f, h, j, l, q, dd, ee, gg, ii, jj, kk, ll and zz vary from 1 to 100; Bm is any bioactive peptide, protein, cell, oligosaccharide, glycopeptide, peptidomimetic, drug, drug mimic, hormone, metal chelating agent, radioactive or nonradioactive metal complex, or echogenic agent; Z¹ is selected from the group consisting of -(CH₂)_r-CO₂H, -(CH₂)_r-OH, -(CH₂)_r-NH₂, -CH₂-(CH₂OCH₂)_s-CH₂-CO₂H, -CH₂-(CH₂OCH₂)_s-CH₂-OH, -CH₂-(CH₂OCH₂)_s-CH₂-NH₂, -(CH₂)_t-CONH-Dm, -CH₂-

$(\text{CH}_2\text{OCH}_2)_o\text{-CH}_2\text{-CONH-Dm}$, $-(\text{CH}_2)_x\text{-NHCO-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_o\text{-CH}_2\text{-NHCO-Dm}$, $-(\text{CH}_2)_w\text{-N(R}^{14})\text{-(CH}_2)_x\text{-CONH-Dm}$, $(\text{CH}_2)_{mm}\text{-N(R}^{35})\text{-(CH}_2)_{nn}\text{-NHCO-Dm}$, $-(\text{CH}_2)_y\text{-N(R}^{15})\text{-CH}_2\text{-(CH}_2\text{OCH}_2)_z\text{-CH}_2\text{-CONH-Dm}$, $-(\text{CH}_2)_{uu}\text{-N(R}^{39})\text{-CH}_2\text{-(CH}_2\text{OCH}_2)_{vv}\text{-CH}_2\text{-NHCO-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_{ww}\text{-CH}_2\text{-N(R}^{40})\text{-(CH}_2)_{xx}\text{-CONH-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_{oo}\text{-CH}_2\text{-N(R}^{36})\text{-(CH}_2)_{pp}\text{-NHCO-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_{qq}\text{-CH}_2\text{-N(R}^{37})\text{-CH}_2\text{-(CH}_2\text{OCH}_2)_t\text{-CH}_2\text{-CONH-Dm}$ or $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_{ss}\text{-CH}_2\text{-N(R}^{38})\text{-CH}_2\text{-(CH}_2\text{OCH}_2)_t\text{-CH}_2\text{-NHCO-Dm}$; r , t , v , w , x , y , mm , nn , pp , uu and xx vary from 1 to 10, and o , s , u , z , oo , qq , rr , ss , tt , vv and ww vary from 1 to 100; and Dm is any bioactive peptide, antibody, antibody fragment, oligosaccharide, drug, drug mimic, glycomimetic, glycopeptide, peptidomimetic, hormone, and the like; R^2 to R^9 may be the same or different and are selected from the group consisting of hydrogen, $\text{C}_1\text{-C}_{10}$ alkyl, $\text{C}_1\text{-C}_{10}$ aryl, hydroxyl, $\text{C}_1\text{-C}_{10}$ polyhydroxyalkyl, $\text{C}_1\text{-C}_{10}$ alkoxy, amino, $\text{C}_1\text{-C}_{10}$ aminoalkyl, cyano, nitro, or halogen.

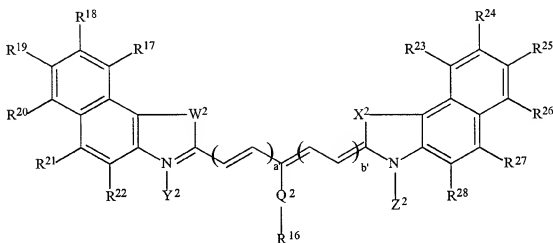
2. The composition of claim 1 wherein a and b vary from 0 to 3; Q^1 is a single bond; R^1 to R^9 are hydrogens; W^1 and X^1 are $-\text{CR}^{10}\text{R}^{11}$; Y^1 is $-(\text{CH}_2)_f\text{-CONH-Bm}$, $-(\text{CH}_2)_k\text{-NHCO-Bm}$, or $-(\text{CH}_2)_m\text{-N(R}^{14})\text{-(CH}_2)_n\text{-CONH-Bm}$; Z^1 is $-(\text{CH}_2)_f\text{-OH}$, $-(\text{CH}_2)_f\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-NH}_2$, $-(\text{CH}_2)_f\text{-CONH-Dm}$, $-(\text{CH}_2)_f\text{-NHCO-Dm}$, $-(\text{CH}_2)_w\text{-N(R}^{14})\text{-(CH}_2)_x\text{-CONH-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_z\text{-CH}_2\text{-OH}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_z\text{-CH}_2\text{-CO}_2\text{H}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_z\text{-CH}_2\text{-NH}_2$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_z\text{-CH}_2\text{-CONH-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_o\text{-CH}_2\text{-NHCO-Dm}$, or $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_{ww}\text{-CH}_2\text{-N(R}^{40})\text{-(CH}_2)_{xx}\text{-CONH-Dm}$; and Bm is a tumor specific biomolecule or drug mimic selected from peptides or oligosaccharides containing 2-50 monomer units, somatostatin, bombesin, neurotensin, cholecystokinin and vasoactive intestinal polypeptide.

3. The composition of claim 1 wherein a and b are 3; Q^1 is a single bond; R^1 to R^9 are hydrogens; W^1 and X^1 are $-\text{C}(\text{CH}_3)_2$; Y^1 is $-(\text{CH}_2)_i\text{-CONH-Bm}$ or $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_j\text{-CH}_2\text{-CONH-Bm}$ wherein i varies from 1 to 4; and Z^1 is $-(\text{CH}_2)_f\text{-CO}_2\text{H}$, $-(\text{CH}_2)_f\text{-CONH-Dm}$, $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_z\text{-CH}_2\text{-CO}_2\text{H}$ or $-\text{CH}_2\text{-(CH}_2\text{OCH}_2)_u\text{-CH}_2\text{-CONH-Dm}$, wherein r and t vary from 1-4; and Bm is a tumor specific biomolecule selected from the group consisting of Octreotate and Octreotide.

4. A method of performing a diagnostic or therapeutic procedure which comprises administering to an individual an effective amount of the composition of claim 1.

- 1 5. A method of performing a diagnostic or therapeutic procedure which comprises
2 administering to an individual an effective amount of the composition of claim 2.
- 1 6. A method of performing a diagnostic or therapeutic procedure which comprises
2 administering to an individual an effective amount of the composition of claim 3.
- 1 7. The method according to any one of claims 4-6 wherein said procedure utilizes light of
2 wavelength in the region of 350-1300 nm.
- 1 8. The method according to any one of claims 4-6 wherein said diagnostic procedure is optical
2 tomography.
- 1 9. The method according to any one of claims 4-6 wherein said diagnostic procedure is
2 fluorescence endoscopy.
- 1 10. The method according to any one of claims 4-6 further comprising monitoring a blood
2 clearance profile of said bioconjugate invasively or non-invasively by fluorescence,
3 absorbance or light scattering wherein light of wavelength in the region of 350-1300 nm is
4 utilized.
- 1 11. The method according to any one of claims 4-6 further comprising a step of imaging or
2 therapy wherein said step of imaging or therapy is selected from an absorption, light
3 scattering, photoacoustic, or sonofluorescence technique.
- 1 12. The method according to any one of claims 4-6 wherein said procedure comprises
2 diagnosing atherosclerotic plaques or blood clots.
- 1 13. The method according to any one of claims 4-6 wherein said procedure comprises
2 administering localized therapy.

14. The method according to any one of claims 4-6 wherein said therapeutic procedure comprises photodynamic therapy.
15. The method according to any one of claims 4-6 wherein said therapeutic procedure comprises laser assisted guided surgery (LAGS) for detection of small micrometastases.
16. A composition comprising of indocyanine bioconjugates of the general formula



wherein a' and b' vary from 0 to 5; W² and X² may be the same or different and are selected from the group consisting of -CR¹⁰R¹¹, -O-, -NR¹², -S-, or -Se; Q² is a single bond or is selected from the group consisting of -O-, -S-, -Se-, and -NR¹³; R¹⁶, R¹⁰ to R¹⁵, and R²⁹-R⁴⁰ may be the same or different and are selected from the group consisting of hydrogen, C₁-C₁₀ alkyl, C₁-C₁₀ aryl, C₁-C₁₀ alkoxy, C₁-C₁₀ polyalkoxyalkyl, -CH₂(CH₂OCH₂)_c-CH₂-OH, C₁-C₂₀ polyhydroxyalkyl, C₁-C₁₀ polyhydroxyaryl, C₁-C₁₀ aminoalkyl, -(CH₂)_d-CO₂H, -(CH₂)_e-CONH-Bm, -CH₂-(CH₂OCH₂)_f-CH₂-CONH-Bm, -(CH₂)_g-NHCO-Bm, -CH₂-(CH₂OCH₂)_h-CH₂-NHCO-Bm, -(CH₂)_{yy}-OH or -CH₂-(CH₂OCH₂)_{zz}-CH₂-OH; Y² is selected from the group consisting of -(CH₂)_i-CONH-Bm, -CH₂-(CH₂OCH₂)_j-CH₂-CONH-Bm, -(CH₂)_k-NHCO-Bm, -CH₂-(CH₂OCH₂)_l-CH₂-NHCO-Bm, -(CH₂)_m-N(R¹⁴)-(CH₂)_n-CONH-Bm, (CH₂)_m-N(R²⁹)-(CH₂)_{bb}-NHCO-Bm, -(CH₂)_p-N(R¹⁵)-CH₂-(CH₂OCH₂)_q-CH₂-CONH-Bm, -(CH₂)_{cc}-N(R³⁰)-CH₂-(CH₂OCH₂)_{dd}-CH₂-NHCO-Bm, -CH₂-(CH₂OCH₂)_{ee}-CH₂-N(R³¹)-(CH₂)_n-CONH-Bm, -CH₂-(CH₂OCH₂)_{ff}-CH₂-N(R³²)-(CH₂)_{hh}-NHCO-Bm, -CH₂-(CH₂OCH₂)_{ii}-CH₂-N(R³³)-CH₂-(CH₂OCH₂)_{jj}-CH₂-CONH-Bm or -CH₂-(CH₂OCH₂)_{kk}-CH₂-N(R³⁴)-CH₂-(CH₂OCH₂)_{ll}-CH₂-NHCO-Bm; d, e, g, i, k, m, n, p, aa, bb, cc, ff, hh and yy vary from 1 to 10; c, f, h, j, l, q, dd,

ee, gg, ii, jj, kk, ll and zz vary from 1 to 100; Bm is any bioactive peptide, protein, cell, oligosaccharide, glycopeptide, peptidomimetic, drug, drug mimic, hormone, metal chelating agent, radioactive or nonradioactive metal complex, or echogenic agent; Z^2 is selected from the group consisting of $-(CH_2)_f-CO_2H$, $-(CH_2)_f-OH$, $-(CH_2)_f-NH_2$, $-(CH_2)(CH_2OCH_2)_s-CH_2-CO_2H$, $-(CH_2)(CH_2OCH_2)_s-CH_2-OH$, $-(CH_2)(CH_2OCH_2)_s-CH_2-NH_2$, $-(CH_2)_k-CONH-Dm$, $-(CH_2)(CH_2OCH_2)_u-CH_2-CONH-Dm$, $-(CH_2)_l-NHCO-Dm$, $-(CH_2)(CH_2OCH_2)_o-CH_2-NHCO-Dm$, $-(CH_2)_w-N(R^{14})-(CH_2)_x-CONH-Dm$, $(CH_2)_{mm}-N(R^{35})-(CH_2)_{nn}-NHCO-Dm$, $-(CH_2)_l-N(R^{15})-CH_2-(CH_2OCH_2)_t-CH_2-CONH-Dm$, $-(CH_2)_{uu}-N(R^{39})-CH_2-(CH_2OCH_2)_{vv}-Cl_2-NHCO-Dm$, $-(CH_2)(CH_2OCH_2)_{ww}-CH_2-N(R^{40})-(CH_2)_{xx}-CONH-Dm$, $-(CH_2)(CH_2OCH_2)_{oo}-CH_2-N(R^{36})-(CH_2)_{pp}-NHCO-Dm$, $-(CH_2)(CH_2OCH_2)_{qq}-CH_2-N(R^{37})-CH_2-(CH_2OCH_2)_n-CH_2-CONH-Dm$ or $-(CH_2)(CH_2OCH_2)_{ss}-CH_2-N(R^{38})-CH_2-(CH_2OCH_2)_n-CH_2-NHCO-Dm$; r, t, v, w, x, y, mm, nn, pp, uu and xx vary from 1 to 10, and o, s, u, z, oo, qq, rr, ss, tt, vv and ww vary from 1 to 100; and Dm is any bioactive peptide, antibody, antibody fragment, oligosaccharide, drug, drug mimic, glycomimetic, glycopeptide, peptidomimetic, hormone, and the like; R^{17} to R^{28} may be the same or different and are selected from the group consisting of hydrogen, C_1-C_{10} alkyl, C_1-C_{10} aryl, hydroxyl, C_1-C_{10} polyhydroxyalkyl, C_1-C_{10} alkoxy, amino, C_1-C_{10} aminoalkyl, cyano, nitro, or halogen.

17. The composition of claim 16 wherein a' and b' vary from 0 to 3; Q^2 is a single bond; R^{16} to R^{28} are hydrogens; W^2 and X^2 are $-CR^{10}R^{11}$; Y^2 is $-(CH_2)_i-CONH-Bm$, $-(CH_2)_i-NHCO-Bm$, or $-(CH_2)_m-N(R^{14})-(CH_2)_n-CONH-Bm$; Z^2 is $-(CH_2)_f-CO_2H$, $-(CH_2)_f-NH_2$, $-(CH_2)_f-OH$, $-(CH_2)_f-CONH-Dm$, $-(CH_2)_v-NHCO-Dm$, $-(CH_2)_w-N(R^{14})-(CH_2)_x-CONH-Dm$, $-(CH_2)(CH_2OCH_2)_s-CH_2-CO_2H$, $-(CH_2)(CH_2OCH_2)_s-CH_2-NH_2$, $-(CH_2)(CH_2OCH_2)_s-CH_2-OH$, $-(CH_2)(CH_2OCH_2)_u-CH_2-CONH-Dm$, $-(CH_2)(CH_2OCH_2)_o-CH_2-NHCO-Dm$, $-(CH_2)(CH_2OCH_2)_{ww}-CH_2-N(R^{40})-(CH_2)_{xx}-CONH-Dm$; and Bm is a tumor specific biomolecule or drug mimic selected from the group consisting of peptides and oligosaccharides containing 2-50 monomer units.

18. The composition of claim 16 wherein a' and b' are 3; Q^2 is a single bond; R^{16} to R^{28} are hydrogens; W^2 and X^2 are $-C(CH_3)_2$; Y^2 is $-(CH_2)_i-CONH-Bm$ or $-(CH_2)(CH_2OCH_2)_j-CH_2-CONH-Bm$ wherein i varies from 1 to 4; and Z^2 is $-(CH_2)_f-CO_2H$, $-(CH_2)_f-CONH-Dm$, $-(CH_2)(CH_2OCH_2)_s-CH_2-CO_2H$ or $-(CH_2)(CH_2OCH_2)_u-CH_2-CONH-Dm$, wherein r and t vary from

- 5 1-4; and Bm is a tumor specific biomolecule selected from the group consisting of Octreotate
6 and Octreotide.
- 1 19. A method of performing a diagnostic or therapeutic procedure which comprises
2 administering to an individual an effective amount of the composition of claim 16.
- 1 20. A method of performing a diagnostic or therapeutic procedure which comprises
2 administering to an individual an effective amount of the composition of claim 17.
- 1 21. A method of performing a diagnostic or therapeutic procedure which comprises
2 administering to an individual an effective amount of the composition of claim 18.
- 1 22. The method according to any one of claims 19-21 wherein said procedure utilizes light of
2 wavelength in the region of 350-1300 nm.
- 1 23. The method according to any one of claims 19-21 wherein said diagnostic procedure is
2 optical tomography.
- 1 24. The method according to any one of claims 19-21 wherein said diagnostic procedure is
2 fluorescence endoscopy.
- 1 25. The method according to any one of claims 19-21 further comprising monitoring a blood
2 clearance profile of said bioconjugate invasively or non-invasively by fluorescence,
3 absorbance or light scattering wherein light of wavelength in the region of 350-1300 nm is
4 utilized.
- 1 26. The method according to any one of claims 19-21 further comprising a step of imaging or
2 therapy wherein said step of imaging or therapy is selected from an absorption, light
3 scattering, photoacoustic, or sonofluorescence technique.
- 1 27. The method according to any one of claims 19-21 wherein said procedure comprises
2 diagnosing atherosclerotic plaques or blood clots.

- 1 28. The method according to any one of claims 19-21 wherein said procedure comprises
2 administering localized therapy.
- 1 29. The method according to any one of claims 19-21 wherein said therapeutic procedure
2 comprises photodynamic therapy.
- 1 30. The method according to any one of claims 19-21 wherein said therapeutic procedure
2 comprises laser assisted guided surgery (LAGS) for detection of small micrometastases.
- 1 31. A method of preventing *in vivo* or *in vitro* fluorescence quenching which comprises adding
2 one to fifty percent of a biocompatible organic solvent to a diagnostic or therapeutic
3 composition of dye molecules.
- 1 32. The method of claim 31 wherein said dye molecules are dissolved in a medium comprising
2 one to 50 percent dimethyl sulfoxide (DMSO).

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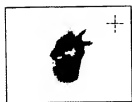


FIG. 1A

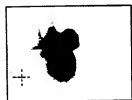


FIG. 1B

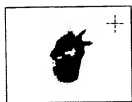


FIG. 1C

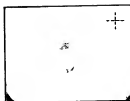


FIG. 1D

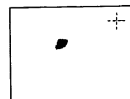


FIG. 1E

FIG. 1F

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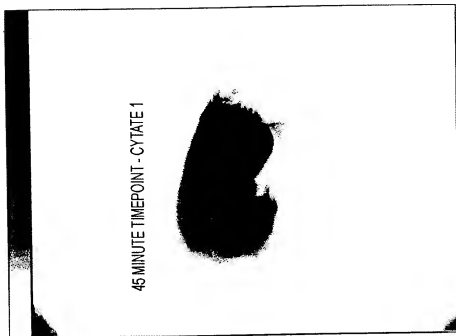


FIG. 2B

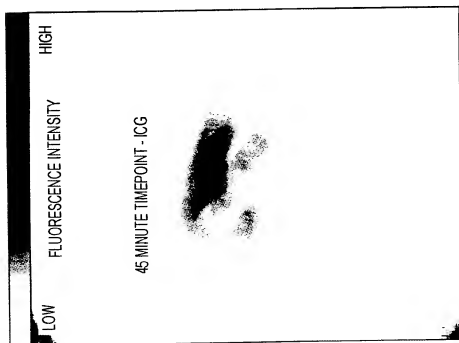


FIG. 2A

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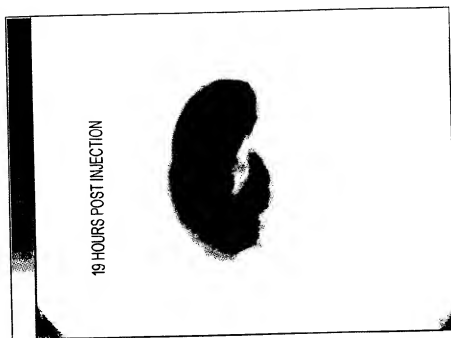


FIG. 3B

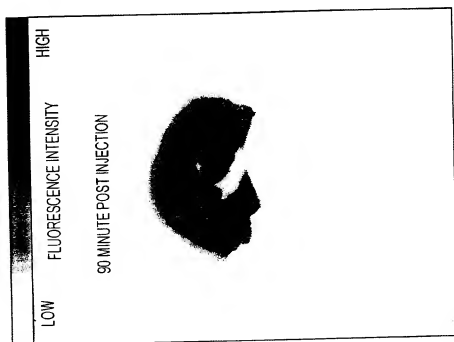


FIG. 3A

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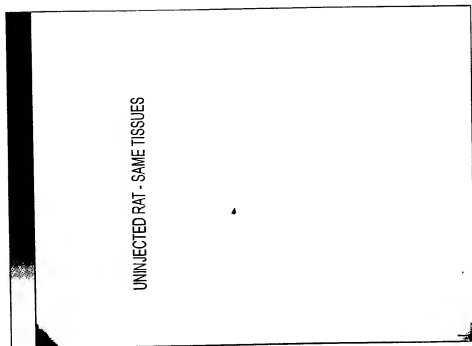


FIG. 4B

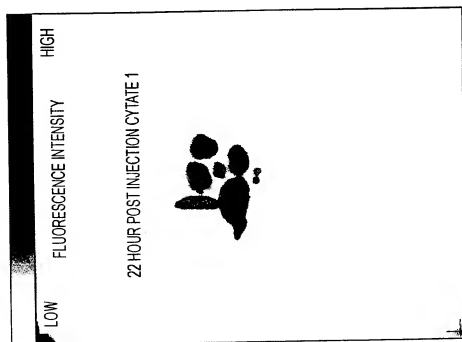


FIG. 4A

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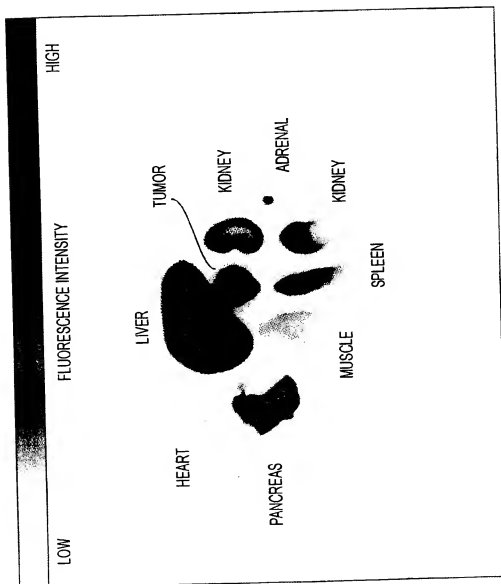
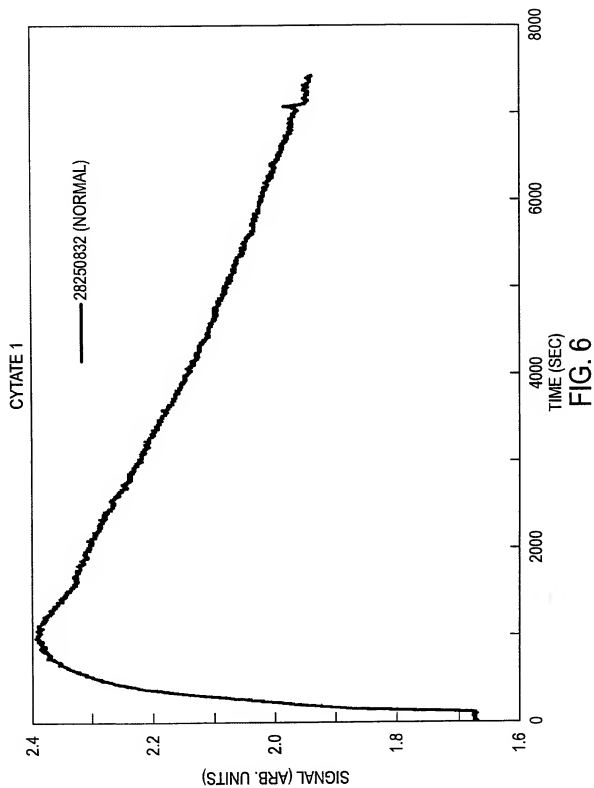
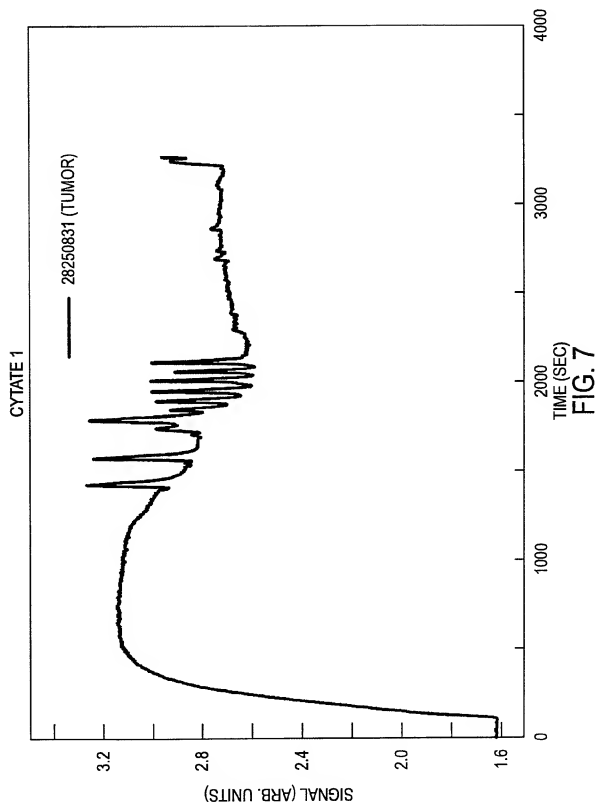


FIG. 5

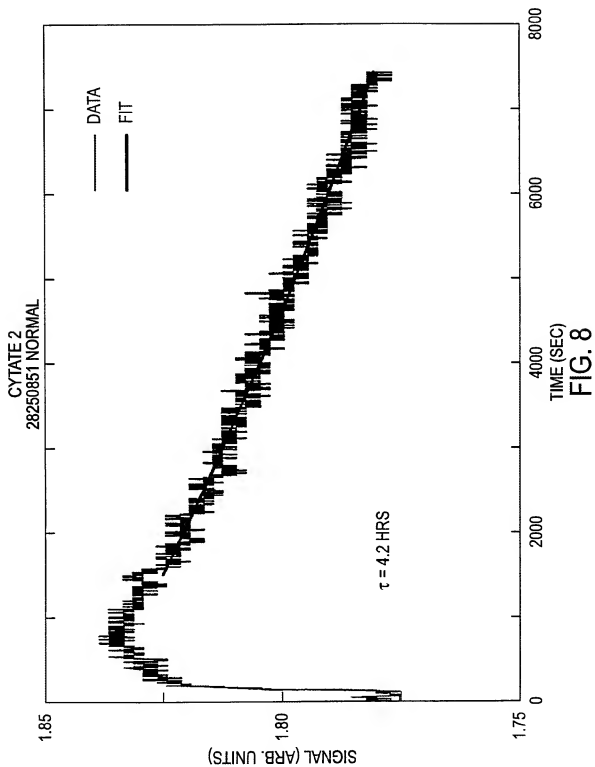
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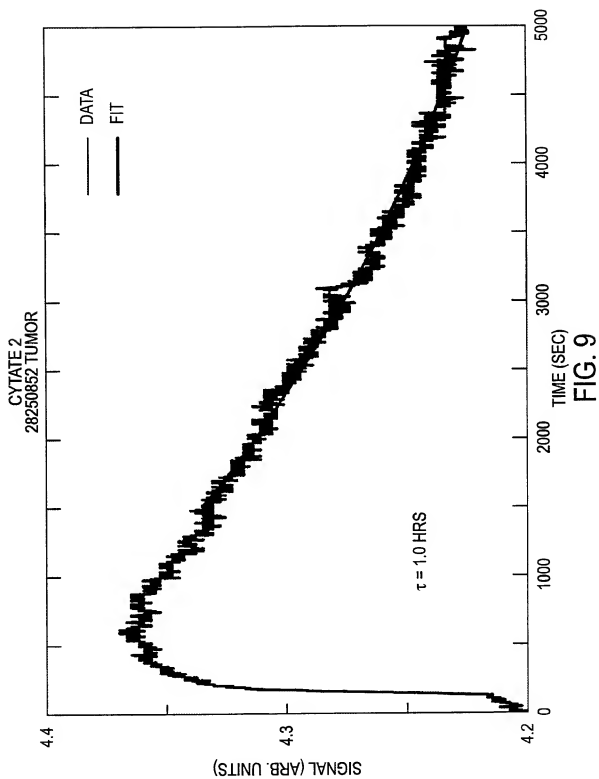
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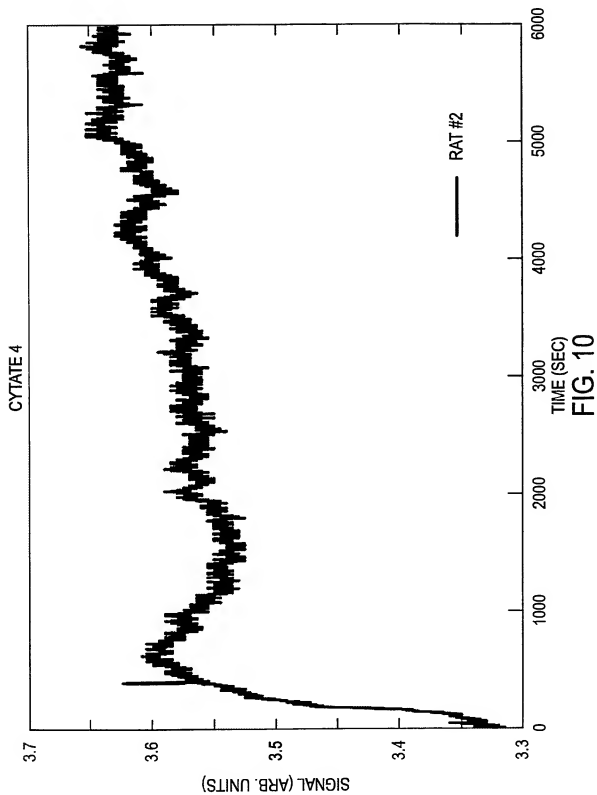
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10/10



SEQUENCE LISTING

<110> Achilefu, Samuel
Dorshow, Richard B.
Buga, Joseph E.
Rajagopalan, Raghavan
Mallinckrodt Inc.

<120> NOVEL CYANINE AND INDOCYANINE DYE BIOCONJUGATES FOR
BIOMEDICAL APPLICATIONS

<130> 1668-286-PCT

<140> Not yet assigned
<141> 2000-04-26

<150> U.S. 60/135,060
<151> 1999-05-20

<150> U.S. 09/325,769
<151> 1999-06-04

<160> 8

<170> PatentIn Ver. 2.0

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<220>
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1 5

<210> 6
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<220>
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<220>
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<222> (6)
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<220>
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<220>
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1 5

<210> 7
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(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 November 2000 (30.11.2000)

PCT

(10) International Publication Number
WO 00/71162 A3

(51) International Patent Classification⁷: **A61K 41/00**,
49/00

St. Charles, MO 63303 (US). RAJAGOPALAN, Raghavan; 13031 Vinson Court, Maryland Heights, MO 63043 (US).

(21) International Application Number: PCT/US00/11060

(22) International Filing Date: 26 April 2000 (26.04.2000)

(74) Agents: REPPER, George, R. et al.; Rothwell, Figg, Ernst & Manbeck, P.C., Suite 701 East, 555 13th Street N.W., Columbia Square, Washington, DC 20004 (US).

(25) Filing Language: English

(81) Designated States (*national*): AU, CA, JP.

(26) Publication Language: English

(84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

(30) Priority Data:
60/135,060 20 May 1999 (20.05.1999) US
09/325,769 4 June 1999 (04.06.1999) US

Published:
— With international search report.

(71) Applicant: MALLINCKRODT INC. [US/US]; 675 McDonnell Boulevard, P.O. Box 5840, St. Louis, MO 63134 (US).

(88) Date of publication of the international search report:
5 July 2001

(72) Inventors: ACHILEFU, Samuel; 3424 San Sevilla Court, Bridgeton, MO 63044 (US). DORSHOW, Richard, Bradley; 11977 Niehaus Lane, St. Louis, MO 63146 (US). BUGAJ, Joseph, Edward; 2916 Kettering Drive,

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 00/71162 A3

(54) Title: CYANINE AND INDOCYANINE DYE BIOCONJUGATES FOR BIOMEDICAL APPLICATIONS

(57) Abstract: Dye-peptide conjugates useful for diagnostic imaging and therapy are disclosed. The dye-peptide conjugates include several cyanine dyes with a variety of bis- and tetrakis (carboxylic acid) homologues. The small size of the compounds allows more favorable delivery to tumor cells as compared to larger molecular weight imaging agents. The various dyes are useful over the range of 350-1300 nm, the exact range being dependent upon the particular dye. Use of dimethylsulfoxide helps to maintain the fluorescence of the compounds. The molecules of the invention are useful for diagnostic imaging and therapy, in endoscopic applications for the detection of tumors and other abnormalities and for localized therapy, for photoacoustic tumor imaging, detection and therapy, and for sonofluorescence tumor imaging, detection and therapy.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/11060

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K41/00 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EMBASE, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 97 33620 A (GOETZ ALWIN ;PFEIFFER ULRICH (DE); PUEHLER GABRIELA (DE); PULSION) 18 September 1997 (1997-09-18) claims ---	1-32
X	WO 00 41725 A (CHEN JAMES ;LIGHT SCIENCES LTD (US)) 20 July 2000 (2000-07-20) claims ---	1
E	WO 00 41726 A (CHEN JAMES ;LIGHT SCIENCES LTD (US)) 20 July 2000 (2000-07-20) claims --- -/-	1

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex

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O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

19 January 2001

Date of mailing of the international search report

06/02/2001

Name and mailing address of the ISA

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NL - 2280 HV Rijswijk
Tel (+31-70) 340-2340, Tx. 31 651 epo nl,
Fax (+31-70) 340-3016

Authorized officer

Berte, M

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/US 00/11060

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	US 5 453 505 A (LEE LINDA G ET AL) 26 September 1995 (1995-09-26) column 2, line 64 -----	1
Y		1-32
X	DE 196 49 971 A (DIAGNOSTIKFORSCHUNG INST) 28 May 1998 (1998-05-28) page 5, line 54 - line 65; claims -----	1-32
X	WO 96 17628 A (DIAGNOSTIKFORSCHUNG INST ;LICHIA KAI (DE); RIEFKE BJOERN (DE); SEMM) 13 June 1996 (1996-06-13) page 17, line 23 - line 35; claims page 18, line 1 - line 25 -----	1-32
X	SAVITSKY, A. P. ET AL: "Photophysical properties of protein conjugates with PDT photosensitizers" PROC. SPIE-INT. SOC. OPT. ENG. (1993), 1922(LASER STUDY OF MACROSCOPIC BIOSYSTEMS), 245-54 , XP000978928 tables 1,6 -----	1-32
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P,X	WO 99 51284 A (ACHILEFU SAMUEL I ;DORSHOW RICHARD B (US); BUGAJ JOSEPH E (US); MA) 14 October 1999 (1999-10-14) claims -----	1-32

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claim(s) 4-15, 16-32 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
Although claims 4-15, 16-32 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 00/11060

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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